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Original Article

Outcomes of anti-Müllerian hormone-tailored ovarian stimulation protocols in *in vitro* fertilization/intracytoplasmic sperm injection cycles in women of advanced age

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ABSTRACT

Objective: We aimed to compare the outcomes of *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatments in women of advanced age (>40 years) using anti-Müllerian hormone (AMH)-tailored ovarian stimulation protocols versus conventional protocols based on antral follicle count (AFC).
Materials and Methods: We retrospectively reviewed 210 women who underwent IVF/ICSI cycles: 116 women underwent stimulation protocols that were tailored to their AMH levels, whereas 94 women received treatment using conventional stimulation protocols based on AFC as the ovarian reserve marker.
Results: The following parameters were significantly higher in the AMH-tailored group than in the conventional group: initial and total doses (IU) of recombinant follicle-stimulating hormone (rFSH) used for stimulation (514.2 ± 137.9 vs. 452.3 ± 135.3, $p = 0.001$; 4713.8 ± 1618.8 vs. 4047.2 ± 1366.0, $p = 0.007$, respectively), ovum pick-up rate (OPU; 88.8% vs. 75.5%, $p = 0.016$), serum estradiol (E2) level on the day of human chorionic gonadotropin (hCG) administration (1818.5 ± 1422.4 vs. 1394.0 ± 929.0 pg/mL, $p = 0.028$), number of oocytes retrieved (7.4 ± 5.1 vs. 5.5 ± 3.4, $p = 0.007$), number of embryos per case (4.2 ± 3.2 vs. 3.3 ± 2.5, $p = 0.048$), clinical pregnancy rates (22.4% vs. 8.5%, $p = 0.008$), implantation rates (13.1% vs. 3.9%, $p = 0.001$), and live birth rates per cycle (15.5% vs. 6.4%, $p = 0.049$).
Conclusion: Individualized controlled ovarian stimulation (COS) protocols tailored to patients' AMH levels may improve the pregnancy rate, implantation rate, and live birth rate in women of advanced age undergoing IVF/ICSI compared with those receiving conventional stimulation protocols.

Introduction

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein produced by the granulosa cells of preantral and antral ovarian follicles. In the past, AMH has played a significant role in infertility assessment and treatment because it is a more accurate marker for predicting ovarian response to controlled ovarian stimulation (COS) than age or levels of Day 3 follicle-stimulating hormone (FSH), estradiol (E2), and inhibin B [1–3]. Before *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatments are initiated, AMH levels can help guide clinicians in counseling their patients about the risks and benefits of treatment and the likelihood of success, as well as allowing them to individualize treatment strategy according to the anticipated ovarian reserve. AMH levels are also correlated with the onset of menopause; however, its value is much more limited in the accurate prediction of the age of menopause [4]. In several reviews and meta-analyses, AMH measurement prior to ovarian stimulation has been associated with accurate prediction of ovarian over-response; therefore, choosing a

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stimulation strategy and adjusting the FSH dose according to the patient's serum AMH level may help reduce the incidence of ovarian hyperstimulation syndrome (OHSS) [1–3,5,6]. However, the utility of AMH levels for predicting poor ovarian response has been questioned, and evidence supporting the role of AMH in predicting pregnancy outcomes is scarce [5–7]. Thus, the aim of this study was to compare the outcomes of IVF/ICSI between women of advanced age using AMH-tailored stimulation protocols and conventional stimulation protocols based on antral follicle count (AFC) as the ovarian reserve marker.

Material and methods

Study participants

We retrospectively reviewed and analyzed the medical records of all patients aged > 40 years who received IVF/ICSI treatment at the Infertility Division of the Department of Obstetrics and Gynecology at MacKay Memorial Hospital in Taipei, Taiwan, between January 1, 2006 and September 30, 2011. The study protocol was approved by the Institutional Review Board of MacKay Memorial Hospital. The inclusion criteria for the reference population were patients who underwent a cycle of IVF, with or without ICSI, using fresh embryos, during the study period. The exclusion criteria were the presence of any of the following conditions: (1) infertility due to a uterine factor (e.g., thin endometrium, endometrial synechiae); (2) cancer; and (3) systemic disease (e.g., diabetes mellitus, thyroid disease, autoimmune disease).

Study design

Participants were divided into two groups according to the availability of AMH data. The control group comprised women who were treated with a conventional protocol of ovarian stimulation using chronological age and AFC as markers of the ovarian reserve, because AMH data was not obtained prior to beginning ovarian stimulation. The starting dose of gonadotropins for the control group was determined according to AFC with a baseline dose of 450 IU if AFC $\leq$ 5 or decrease dose to 150 IU if AFC > 15.

The average time required to receive AMH level test results in our hospital is 2 weeks; therefore, AMH data for women in the control group was usually not obtained due to the time constraints of patients who live far away from the hospital or abroad, or who did not have a chance to obtain an AMH-level test prior to initiating treatment. The study group consisted of women whose basal AMH levels were measured within 3 months prior to treatment, and whose starting dose of gonadotropins was tailored to their serum levels of AMH of those < 1.0 receiving a 600-IU dose of FSH, those $\geq$ 1.0 and < 1.2 receiving a 525-IU dose of FSH, those $\geq$ 1.2 and < 1.5 receiving a 450-IU dose of FSH, those $\geq$ 1.5 and < 2 receiving a 300-IU dose of FSH, those $\geq$ 2 and < 5 receiving a dose of 225-IU, and those $\geq$ 5 receiving a dose of 150 IU.

Poor response to ovarian stimulation, which resulted in cycle cancelation, was defined as a serum E2 level of $\leq$ 500 pg/mL and $\leq$ two follicles > 16 mm seen on transvaginal ultrasonography on the day of human chorionic gonadotropin (hCG) administration. The main outcomes compared included the initial dose of recombinant FSH (rFSH), total dose of rFSH, duration of stimulation, serum E2 level on hCG day, ovum pick-up (OPU) rate, number of oocytes retrieved, number of embryos per case, embryo transfer (ET) rate, number of embryos transferred, clinical pregnancy rate, implantation rate, live birth rate, and abortion rate of women receiving AMH-tailored stimulation protocols and those undergoing conventional stimulation protocols.

COS

Pelvic ultrasonography was used to check for pelvic cavity abnormalities, including ovarian tumors, on Day 2–3 of the menstrual cycle. Patients underwent IVF treatment using a long down-regulation or short flare-up protocol with a gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist protocol. In the GnRH agonist protocol, pituitary suppression was initiated with 1 mg subcutaneous leuprolide acetate (Takeda Pharma GmbH, Stolberg, Germany) beginning on Day 21 of the previous menstrual cycle until the serum levels of E2 fell below 30 pg/mL, and thereafter 0.5 mg leuprolide acetate until hCG day. In the GnRH antagonist protocol, 0.25 mg subcutaneous cetrorelix (Cetrotide; Serono, Baxter Oncology GmbH, Halle, Germany) or 0.25 mg ganirelix acetate (Orgalutran; Schering-Plough, Whitehouse Station, NJ, USA) was administered daily when the follicles were > 14 mm in diameter until hCG day. All patients received rFSH (Gonal-F; Serono Laboratories, Aubonne, Switzerland) and/or human menopausal gonadotropin (Menopur; Ferring GmbH, Kiel, Germany). The dosage of gonadotropin was determined by the age of the patient and the AFC or the AMH levels. The dosage was then adjusted every 2–3 days in accordance with follicle growth. When a leading follicle $\geq$ 18 mm in diameter was detected by ultrasonography, 10,000 IU of hCG (Pregnyl; Schering-Plough, Kenilworth, NJ, USA) or 250 μg of choriogonadotropin-alfa (Ovidrel; Serono, Rome, Italy) was administered, and oocyte retrieval was performed 34–36 hours later. We performed IVF or ICSI with either ejaculated sperm or surgically retrieved sperm. Up to four embryos were transferred into the uterine cavity on Day 2–3 after oocyte retrieval according to the embryo number and quality. Clinical pregnancy was defined as the presence of an intrauterine gestational sac by ultrasonography at approximately 5 weeks of pregnancy.

Serum AMH measurement

AMH levels were measured by enzyme-linked immunosorbent assay kit (Diagnostic Systems Laboratories, Webster, TX, USA). The detection range of the assay was 0.025–15 ng/mL, with the detection limit at 0.017 ng/mL. Values below the detection limit were considered zero. The intra-assay and interassay variation coefficients were 4.6% and 8.0%, respectively. Samples from all participants were obtained via venipuncture and analyzed by the same laboratory (Department of Immunoassay, MacKay Memorial Hospital, Taipei, Taiwan). The samples were processed according to the manufacturer instructions by centrifuging at 1400*g* for 10 minutes to separate cellular contents and debris; the serum was then transferred to sterile polypropylene tubes to be preserved at −70°C until the assay.

Statistical analysis

The main outcome measures were compared using the Chi-square test or Fisher exact test for comparing percentages and a *t* test for comparing mean values. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). A p value $\leq$ 0.05 was considered statistically significant for all measures.

Results

After applying inclusion and exclusion criteria, 210 patients were included; of them, 116 patients comprised the study group (i.e., the AMH-tailored group), whereas 94 patients comprised the control group (i.e., the conventional stimulation group). Table 1 summarizes their characteristics.

Table 1
Characteristics of anti-Müllerian hormone (AMH)-tailored ovarian stimulation in *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles in women of advanced age (> 40 years).

	AMH-tailored group	Conventional stimulation group	*p*
No. of cases	116	94	
Causes of infertility			
1. Male factor	33 (28.4)	30 (31.9)	0.522
2. Tubal factor	31 (26.7)	21 (22.3)	0.65
3. Endometriosis	18 (15.5)	8 (8.5)	0.144
4. Ovulation dysfunction	15 (12.9)	24 (25.5)	0.021
5. Other	19 (16.4)	11 (11.7)	0.428
AMH level (ng/mL), mean ± SD	1.79 ± 1.51	—	—
Use of ICSI	56 (48.3)	35 (37.2)	0.124

Data are presented as *n* (%) unless otherwise indicated.
ICSI = intracytoplasmic sperm injection; SD = standard deviation.

There was no significant difference between the groups except in the number of cases with ovulation dysfunction, which was significantly higher in the conventional stimulation group ($p = 0.021$). Table 2 shows the outcomes in the AMH-tailored group and conventional stimulation group. The initial dose of rFSH selected and the total dose of rFSH used were significantly higher in the AMH-tailored group than in the conventional stimulation group (514.2 ± 137.9 IU vs. 452.3 ± 135.3 IU, $p = 0.001$ and 4713.8 ± 1618.8 IU vs. 4047.2 ± 1366.0 IU, $p = 0.007$). The following parameters were also significantly higher in the AMH-tailored group: OPU rate (88.8% vs. 75.5%, $p = 0.016$), serum E2 level on the day of hCG administration (1818.5 ± 1422.4 pg/mL vs. 1394.0 ± 929.0 pg/mL, $p = 0.028$), number of oocytes retrieved (7.4 ± 5.1 vs. 5.5 ± 3.4, $p = 0.007$), number of embryos per case (4.2 ± 3.2 vs. 3.3 ± 2.5, $p = 0.048$), clinical pregnancy rates (22.4% vs. 8.5%, $p = 0.008$), implantation rates (13.1% vs. 3.9%, $p = 0.001$), and live birth rates per cycle (15.5% vs. 6.4%, $p = 0.049$). There were no significant differences in the duration of stimulation, ET rate, number of transferred embryos per ET, and the abortion rate between the two groups.

Discussion

Individualization of IVF treatment allows clinicians to manage infertile women according to their unique characteristics; this would ideally maximize the clinical pregnancy rate, reduce iatrogenic risks such as OHSS, and minimize the risk of cycle cancelation. In particular, the ability to predict an individual patient's ovarian response to stimulation is very useful for selecting a gonadotropin dosage that is likely to be both effective and safe. The decision making is usually empirical and based on the clinician's preference, if no previous cycle has been performed [8].

Serum AMH levels seem to be a better marker for predicting ovarian response to COS than either patient age or serum levels of FSH, E2, and inhibin B. In the clinical setting, the intercycle and intracycle variability in serum AMH levels is considered low enough to permit random timing of AMH measurement during the menstrual cycle [1–3,9–11]. While AFC is also a useful marker of ovarian response to COS [2,12], it may be a less reliable measure due to two logistical factors: (1) the limited window of time in which to perform ultrasonographic evaluations of antral follicles; and (2) the variable skills of the operators performing the baseline evaluations.

Several studies have indicated the potential benefits of using AMH to individualize treatment strategies for COS. Nelson et al [6] reported a prospective cohort study of 538 patients in two centers with differential COS strategies based on a centralized AMH measurement. Their results showed that the use of circulating AMH levels to individualize treatment strategies for COS may result in reduced clinical risk, optimized treatment burden, and improved pregnancy rates; however, the investigators stated that further

Table 2
Outcomes of anti-Müllerian hormone (AMH)-tailored ovarian stimulation in *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles in women of advanced age (> 40 years).

	AMH-tailored group	Conventional stimulation group	*p*
No. of cases	116	94	
Initial rFSH dose/d (IU)	514.2 ± 137.9	452.3 ± 135.3	0.001
Total rFSH dose (IU)	4713.8 ± 1618.8	4047.2 ± 1366.0	0.007
Stimulation day	10.2 ± 2.0	10.1 ± 2.3	0.824
E2 level on trigger day (pg/mL)	1818.5 ± 1422.4	1394.0 ± 929.0	0.028
OPU rate (%)	88.8	75.5	0.016
Cycle cancellation rate (%)	11.2	24.5	0.016
Embryo no./case	4.2 ± 3.2	3.3 ± 2.5	0.048
Transferred embryo no./case	3.1 ± 1.1	2.9 ± 1.3	0.293
Embryo transfer rate (%)	75.9	66.0	0.126
Clinical pregnancy rate/cycle (%)	22.4	8.5	0.008
Implantation rate (%)	13.1	3.9	0.001
Abortion rate (%)	26.9	12.5	0.645
Ectopic pregnancy rate (%)	3.8	12.5	0.421
Live birth rate/cycle (%)	15.5	6.4	0.049

Data are presented as mean ± SD or %, unless otherwise indicated.
E2 = estradiol; OPU = ovum pick-up; rFSH = recombinant follicle-stimulating hormone; SD = standard deviation.

prospective randomized studies are needed to validate their findings. Moreover, the results of the present study are supported by those of a recent large retrospective study by Yates et al [7]. They evaluated 769 women aged < 40 years undergoing a first cycle of IVF using fresh embryos, and found that women who received individualized AMH-tailored COS protocols had significantly higher rates of fertilization, ET, pregnancy, and live birth than those who underwent conventional stimulation protocols. Furthermore, tailoring stimulation protocols to AMH levels decreased the incidence of adverse outcomes such as OHSS and failed fertilization, and reduced the financial burden associated with assisted reproduction. Unfortunately, the patients in each group of the study were not treated during the same period of time, and therefore the outcomes of these IVF cycles may have varied simply because the quality and performance of IVF management improved with time.

Arce et al [13] also noted a positive association between serum AMH concentrations and the clinical outcome in fresh IVF cycles. They collected data from 749 women, aged 21–34 years, with a primary diagnosis of unexplained infertility or mild male factor infertility, who had serum FSH levels of 1–12 IU/L and AFC $\geq$ 10 in a GnRH antagonist cycle with compulsory single-blastocyst transfer. When comparing patients with AMH levels above and below the 50th percentile, both the ongoing pregnancy rate (32% vs. 23%, $p = 0.006$) and the live birth rate (31% vs. 23%, $p = 0.022$) were significantly higher in patients with high AMH levels. The positive association between AMH levels and clinical outcomes reflected the strong correlation with oocyte yield and the availability of more oocytes and more embryos, at least in younger patients. In the present study, the study population comprised women of advanced age from the same period of time. To the best of our knowledge, ours is the first study to report the outcomes of AMH-tailored stimulation protocols in women aged > 40 years.

A key factor determining the outcome of COS is the selection of the starting dose of gonadotropin. The need for individualizing gonadotropin dosage derives from the assumption that variability in the functional ovarian reserve and the pool of recruitable follicles is very wide; thus, a standard fixed dose of gonadotropin may not be suitable for all cases [8]. Lee et al [14] reported that women aged > 40 years with an AMH concentration in the middle third (0.49~1.22 ng/mL) or upper third ($\geq$ 1.23 ng/mL) had increased clinical pregnancy rates, decreased cycle cancelation rates, and increased numbers of eggs retrieved and embryos transferred than women in the lower third ($\leq$ 0.48 ng/mL). We speculate that increasing the starting gonadotropin dosage used in patients in the lower and middle thirds of AMH concentration might have resulted in improved clinical pregnancy rates comparable with those in the upper third.

In the present study, the mean initial dose of rFSH and the mean total dose of rFSH were both significantly higher in the AMH-tailored stimulation group. We supposed that AFC and AMH were both better ovarian reserve markers. Initially, we only used AFC for initial dose adjustment; a wide variation by different ultrasound operators and miscalculation happened often. Thus, there may be better results from the use of higher doses of gonadotropins in the AMH-tailored group because of the better estimated ovarian reservation evaluation, and this prevents the possibility of unjustified gonadotropin doses. We increased the starting dose and total dose of rFSH in patients with relatively lower levels of AMH with the goal of stimulating more follicles for oocyte retrieval and thus reducing cycle cancelation rates. We calculated the clinical pregnancy rate after excluding the cancellation cases, and it still remained the trend with the better clinical pregnancy rates of 25.2% versus 11.3%, $p = 0.031$ in the study group. Our results indicated that the OPU rate, serum E2 level on trigger day, number of oocytes retrieved, and number of embryos per case were also significantly higher in the AMH-tailored stimulation group. Because a greater number of embryos developed overall, the chances of obtaining high-quality embryos to transfer were improved. These outcomes were also associated with a significantly improved clinical pregnancy rate, implantation rate, and live birth rate per cycle in women aged > 40 years who underwent an AMH-tailored stimulation protocol.

It is our limitation that the small sample size ($n = 210$) of women aged > 40 years undergoing IVF treatment and the retrospective design cannot make a conclusive treatment guideline. The high incidence of ovulation dysfunction in the conventional group may also have a selection bias. In the future, particularly when targeting only a specific group of the population with infertility, a larger sample size and a prospective, randomized controlled design would be preferable.

Ovarian reserve marker assessment, such as AMH and AFC, allows clinicians to set expectations for an individual patient's ovarian response to gonadotropins and to adjust ovarian stimulation strategies accordingly. The study results in the AMH-tailored group, an increased starting dose and total dose of rFSH in patients with relatively low levels of AMH, might help to stimulate more follicles and, consequently, reduce cycle cancelation rates and increase serum E2 levels and the number of oocytes retrieved. In turn, a higher number of oocytes yielded a greater number of embryos to select from and transfer. Thus, individualized COS protocols tailored by AMH level ultimately may improve clinical outcomes such as pregnancy rate, implantation rate, and live birth rate in women of advanced age.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

References

[1] La Marca A, Broekmans FJ, Volpe A, Fauser BC, Macklon NS. Anti-Mullerian hormone (AMH): what do we still need to know? Hum Reprod 2009;24:2264–75.
[2] La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2010;16:113–30.
[3] La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod 2006;21:3103–7.
[4] Dolleman M, Depmann M, Eijkemans MJ, Heimensem J, Broer SL, van der Stroom EM, et al. Anti-Mullerian hormone is a more accurate predictor of individual time to menopause than mother's age at menopause. Hum Reprod 2014;29:584–91.
[5] Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles–implications for individualization of therapy. Hum Reprod 2007;22:2414–21.
[6] Nelson SM, Yates RW, Lyall H, Jamieson M, Traynor I, Gaudoin M, et al. Anti-Mullerian hormone-based approach to controlled ovarian stimulation for assisted conception. Hum Reprod 2009;24:867–75.
[7] Yates AP, Rustamov O, Roberts SA, Lim HY, Pemberton PW, Smith A, et al. Anti-Mullerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. Hum Reprod 2011;26:2353–62.
[8] La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. Hum Reprod Update 2014;20:124–40.
[9] Barad DH, Weghofer A, Gleicher N. Comparing anti-Mullerian hormone (AMH) and follicle-stimulating hormone (FSH) as predictors of ovarian function. Fertil Steril 2009;91(4 Suppl.):1553–5.
[10] Sowers M, McConnell D, Gast K, Zheng H, Nan B, McCarthy JD, et al. Anti-Mullerian hormone and inhibin B variability during normal menstrual cycles. Fertil Steril 2010;94:1482–6.
[11] Toner JP, Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: a sea change in determining ovarian reserve using anti-Mullerian hormone. Fertil Steril 2013;99:1825–30.
[12] Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update 2013;19:26–36.

[13] Arce JC, La Marca A, Mirner Klein B, Nyboe Andersen A, Fleming R. Anti-Mullerian hormone in gonadotropin releasing-hormone antagonist cycles: prediction of ovarian response and cumulative treatment outcome in good-prognosis patients. Fertil Steril 2013;99:1644–53.

[14] Lee RK, Wu FS, Lin MH, Lin SY, Hwu YM. The predictability of serum anti-Mullerian level in IVF/ICSI outcomes for patients of advanced reproductive age. Reprod Biol Endocrinol 2011;9:115.